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ALSTON & BIRD LLP

By:_____
ShengFeng Chen

PATENT
Attorney Docket No.: 057436-392276

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Yednock, Ted
Vasquez, Nicki
Bard, Frederique
Seubert, Peter A.

Application No.: 10/544,093

Filed: August 1, 2005

For: ACTIVE IMMUNIZATION TO GENERATE ANTIBODIES TO SOLUBLE A-BETA

Customer No.: 00826

Confirmation No. 6443

Examiner: Gregory S. Emch

Technology Center/Art Unit: 1649

DECLARATION UNDER 37 CFR 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

1. I, Jack Steven Jacobsen, state as follows.

2. I am currently an independent pharmaceutical professional. From 2001 to 2009, I served as Associate Director for Neuroscience, Wyeth Research, Princeton, NJ 08543. Wyeth Research is a division of Wyeth. I understand that Wyeth is a co-assignee of the above-captioned application. A copy of my curriculum vitae is attached.

3. From 2001 to 2004, I and/or others acting under my supervision have performed experiments to test Aβ16-23. Here, I present experiments that show Aβ16-23 consistently generates an immune response and has activity in inhibiting cognitive decline but does not induce a T-cell response against Aβ notwithstanding that it occurs in a region of Aβ (residues 14-30) in which most T-cell epitopes are located. These results

provide evidence that Aβ16-23 is surprisingly advantageous for treatment or effecting prophylaxis of Alzheimer's disease. The details of these experiments are as follows.

4. In the first experiment, I studied the effect of active immunization with Aβ16-23 on contextual memory of the transgenic (Tg2576) mice. The transgenic (Tg2576) mice were heterozygous for the K670N/M671L amyloid precursor protein transgene and all transgenic genotypes were confirmed by PCR and all animals homozygous for the Retinal Degeneration (Rd) mutation were excluded. The background strain consisted of a C57Bl6 and 129SJL cross. Tg2576 mice exhibited cognitive deficits in contextual memory beginning at 14-16 weeks of age. Cognitive deficits were particularly prominent at 20 weeks of age and were maintained up to 65 weeks of age.

5. The testing apparatus for the experiment was as follows. Transgenic mice and wild-type littermate control mice were individually housed for at least 2 weeks prior to any testing and allowed *ad libitum* access to food and water. Contextual Fear Conditioning (CFC) Assay occurred in six 30 x 24 x 21cm operant chambers (Med Associates, Inc.) constructed from aluminum sidewalls and plexiglass ceiling, door and rear wall. Each chamber was equipped with a floor consisting of 36 stainless steel rods through which a foot shock could be administered. In addition, each chamber had 2 stimulus lights, one house light and a solenoid. Lighting, the footshock (US) and the solenoid (CS) were all controlled by a PC running MED-PC software. The chambers were located in a sound isolated room in the presence of red light.

6. I and/or others acting under my supervision conducted Contextual Fear Conditioning (CFC) assays according to the following protocol. Mice (n=8-12/genotype/treatment) were trained and tested on two consecutive days. The Training Phase consisted of placing the mice in the operant chambers, illuminating both the stimulus and houselights and allowing them to explore for 2 minutes. At the end of the two minutes, the auditory cue (2Hz clicking via the solenoid; CS) was presented for 15 seconds. The footshock (US; 1.5 mAmp) was administered for the final 2 seconds of the CS and co-terminated with the CS presentation. This procedure was repeated and 30 seconds after the second foot shock the mice were removed from the chambers and

returned to their home cages. Twenty hours after training, animals were returned to the chambers in which they had previously been trained. Freezing behavior, in the same environment in which they had received the shock ("Context"), was then recorded by the experimenter using time sampling in 10 seconds bins for 5 minutes (30 sample points). Freezing was defined as the lack of movement except that required for respiration. At the end of the 5 minute Context test mice were returned to their homecages. Freezing in the Novel and Cue condition was collected after all of the mice had been tested in Context (~60 minutes after the initial Context test). The novel environment consisted of modifications of the operant chamber including an opaque plexiglass divider from the rear right corner to the front left, a plexiglass floor as well as decreased illumination (houselight only). Mice were placed in the Novel context and time sampling was used to collect freezing scores for 3 minutes (18 sample points). At the end of the 3 minutes, the auditory clicker (CS) was presented for 3 minutes during which freezing was again scored (18 sample points). Freezing scores for each animal were converted to percent freezing for each portion of the test. Memory for the context (Contextual memory) for each animal was obtained by subtracting freezing score in the novel condition (a measure of basal activity) from that observed in the context.

7. The treatment regime for experiment 1 is as follows. Wild-type and Tg2576 mice were administered (1) adjuvant RC529SE alone, (2) CRM_{197} ("CRM") and adjuvant RC529SE, (3) 30 μg Aβ16-23 antigen conjugated with CRM, and adjuvant RC529SE, (4) 50 μg Aβ16-23 antigen conjugated with CRM, and adjuvant RC529SE, or (5) 266 monoclonal antibody and adjuvant RC529SE by intraperitoneal injection at 47 days prior to the training phase of the CFC and followed by booster doses of the same amount at 19 and 7 days prior to the training phase of the CFC. The days described correspond to mice age at 13, 17 and 19 weeks. The training phase of CFC was conducted at day 47, *i.e.*, in 20 week old mice.

8. The results show that Tg2576 mice displayed prominent and significant cognitive deficit reversal when administered Aβ16-23 antigen conjugated with CRM and adjuvant RC529SE. This agent was an effective treatment at both doses tested (30 or 5

μg) (*see* Fig. 1). The adjuvant-only control (*i.e.*, no treatment) Tg2576 mice displayed a significant deficit in contextual-dependent memory (*i.e.* a significant memory impairment status) in comparison with wild-type mice (Fig. 1). As a positive control, monoclonal antibody 266 caused a significant improvement in contextual memory (*i.e.* significant memory deficit reversal) of Tg2576 mice relative to control treatments, and no significant memory impairment with respect to wild-type mice.

9. In the second experiment, I studied the effect of active immunization with Aβ16-23 on contextual memory of the pre-plaque-forming PSAPP transgenic mice. The transgenic PSAPP mice over-expressed mutant forms of both APP (e.g. Swedish-type mutant APP (hAPpswe)) and PSEN-1 (e.g. mutant human PS1.sub.M146L) (*see* Holcomb et al., (1998) Nat. Med, 4: 97-110). PSAPP mice exhibited age-related development of amyloid plaques that is similar to that observed in Alzheimer's disease. Deposition of Aβ in the frontal cortex and hippocampus of PSAPP mice as early as 3 months of age progresses to cover substantial portions of these areas of the brain at 12 months. Prominent contextual memory deficit and dense accumulation of plaque were particularly prominent at 20 weeks of age.

10. The testing apparatus for the second experiment was the same as described in paragraph (5).

11. I and/or others acting under my supervision conducted Contextual Fear Conditioning (CFC) assays for this experiment according to the protocol described in paragraph (6).

12. The treatment regime for experiment 2 is as follows. Wild-type and PSAPP mice were administered with (1) adjuvant RC529SE alone, (2) CRM and adjuvant RC529SE or (3) Aβ16-23 antigen conjugated with CRM and adjuvant RC529SE by intraperitoneal injection at 47 weeks prior to the training phase of the CFC and followed by booster doses of the same amount at 46, 43, 38, 34, 23, 14, 9, 4 weeks and 1 week prior to the training phase of the CFC. The PSAPP mice for the experiment were

approximately 8-10 weeks of age during the first immunization. The training phase of CFC was conducted at week 47, *i.e.*, in approximately 50-57 week old mice.

13. The results show that cognitive deficits were significantly inhibited when Aβ16-23 antigen conjugated with CRM, and adjuvant RC529SE was administered to PSAPP mice (see Fig. 2). The adjuvant-only adjuvant-only control (*i.e.*, no treatment) and the CRM and adjuvant RC529SE Tg2576 mice displayed a significant deficit in contextual-dependent memory in comparison with wild-type mice (Fig. 2).

14. In the third experiment, I studied the effect of active immunization with Aβ16-23 on contextual memory of the plaque-bearing PSAPP transgenic mice. The PSAPP transgenic mice is as described in paragraph (9).

15. The testing apparatus for the third experiment was the same as described in paragraph (5).

16. I and/or others acting under my supervision conducted Contextual Fear Conditioning (CFC) assays for this experiment according to the protocol described in paragraph (6).

17. The treatment regime for the third experiment is as follows. Wild-type and PSAPP mice were administered (1) adjuvant RC529SE alone, (2) CRM and adjuvant RC529SE, (3) Aβ16-23 antigen conjugated with CRM, and adjuvant RC529SE or (4) 266 monoclonal antibody and adjuvant RC529SE by intraperitoneal injection at 25 weeks prior to the training phase of the CFC and followed by booster doses of the same amount at 21, 16, 11, 7 days and 1 day prior to the training phase of the CFC. The PSAPP mice for the experiment were approximately 12-13 months of age during the first immunization. The training phase of CFC was conducted at week 25, *i.e.*, in approximately 18-19 month old mice.

18. The results show that plaque bearing PSAPP mice displayed significant improvement in contextual memory (*i.e.* significant cognitive deficit reversal) when administered Aβ16-23 antigen conjugated with CRM, and adjuvant RC529SE (*see* Fig.

3). The adjuvant-only (*i.e.*, no treatment) and the CRM and adjuvant RC529SE treated PSAPP mice displayed a significant deficit in contextual-dependent memory in comparison with wild-type mice (Fig. 3). As a positive control, monoclonal antibody 266 also caused a significant improvement in contextual memory of PSAPP mice relative to control treatments.

19. In the fourth experiment, I studied the effect of active immunization with Aβ16-23 on reduction of amyloid plaque formation in PSAPP transgenic mice. The PSAPP transgenic mice for the fourth experiment was the same as described in paragraph (9). In this study, there were 7 groups of ~8-12 week old, Male, PSAPP mice, with approximately 28 Heterzygous transgenic mice (HH) and 20 wild type non-transgenic litermates (WW) mice per group.

20. The immunizations and bleeding methods of PSAPP mice were as follows: Experimental group 1 was immunized with Aβ16-23 conjugated with CRM_{197} and group 2 was immunized with CRM_{197} alone, both on weeks 0, 4, 9, 14, 19 and 24. Group 3 was used as a negative control and was immunized with PBS on the above mentioned weeks. All mice in groups 1-3 were immunized via subcutaneous injection in the rump with a total volume of 200 µl (100 µl in 2 rump sites administered immediately following each other). Groups 1 and 2 were bled on weeks 5, 15 and 19 via tail nick and all groups were bled on week 27 via the retro-orbital route (non-terminal) or cardiac puncture (terminal). . Less than 10% of the mouse's blood volume was collected at each time point (excluding the terminal bleed on week 25). Isoflurane was used as an inhalant anesthetic during retro-orbital bleeding.

21. The histological, histopathological, and morphometry methods for the brains of PSAPP mice were as follows: The mice were sacrificed between 401 and 425 days after birth. The brain of each PSAPP mouse was removed at necropsy, fixed by immersion in 10% neutral buffered formalin, and trimmed by transverse cuts into four pieces. All four pieces were placed in one tissue cassette identified with the animal number, and the tissue in this cassette was processed into paraffin wax using routine histological methods. Three serial microscopic sections of each brain were produced, and

one was stained with each of the following stains: (i). Hematoxylin and eosin for microscopic findings and microscopically-visible plaques (HE) and (ii). Thioflavin-S-stain for amyloid plaques (Thioflavin). A board-certified veterinary pathologist blinded as to treatment and group evaluated all sections microscopically. HE sections were evaluated for findings, and for microscopically-visible plaques (defined as hyaline acellular parenchymal deposits). The number of microscopically-visible plaques in each HE-stained section was quantified by manually counting the number present in one randomly selected field of view in the CA3 of the hippocampus using a 10x microscope objective. Thioflavin sections were evaluated for amyloid-bearing plaques, defined as foci with gold fluorescence when viewed under ultraviolet epifluorescent light. The number of thioflavin-S-positive amyloid-bearing plaques in each Thioflavin-stained section was quantified by manually counting the number present in one randomly selected field of view in the CA3 of the hippocampus. Thioflavin sections were further evaluated for amyloid burden by capturing one photographic image (SNAP image tiff file) of one randomly selected field of view of the CA3 zone of the hippocampus of the brain of each mouse. Morphometry images were captured using ultraviolet epifluorescent light, a 10x microscope objective, a digital camera (Zeiss Axiocam HRc, Zeiss, Inc.), and the camera's matching software (Zeiss Axiovision, Zeiss, Inc.). Each SNAP image tiff file was analyzed individually, in a blinded fashion without knowledge of treatment or group, using Image Pro Plus 5.1 as follows: A. Each image was opened; The number of gold pixels in each image was quantified using the -> Count/Size function of Image Pro Plus 5.1 (MediaCybernetics, Inc., Silver Spring, MD) using the following settings for automated counts and measurements:

Area (range 500-1e+007)
% area (Per Area; range 0-1)
Intensity range selection= manual
Ranges: Histogram based RGB (R=21/255; G=0/255; B=0/255)

Resulting raw data for each image (Raw unmodified data- no deselection) was exported to a Microsoft Excel spreadsheet. Group means for all findings and counts were calculated directly by Microsoft Excel.

22. The results show that immunization of young, pre-plaque-bearing PSAPP mice with Aβ16-23 antigen conjugated with CRM_{197} inhibited amyloid plaque formation compared with immunization with CRM_{197} alone or no immunization treatment (the "no Txt" bars) (*see* Figs. 4A and 4B).

23. In the fifth experiment, I studied the effect of active immunization with Aβ16-23 on induction of T-cell reactivity. In this study, 6-8 week old female, Balb/c mice (5 mice per group) were immunized with various Aβ central epitope peptides conjugated to CRM_{197} plus adjuvant RC529-SE as outlined in Table 1. Positive control animals were immunized with Aβ1-7 conjugated to CRM_{197} plus adjuvant RC529-SE. Mice injected with PBS served as negative controls. Animals were immunized subcutaneously (total volume of 200 μl) on weeks 0, 4, and 12 and bled on weeks 6, 12 and 13. On week 13 spleens were removed for analysis of cell-mediated immune (CMI) responses.

Table 1: Treatment and Number of Animals in each Group

Group #	**Aβ Central Epitopes Peptides Conjugated to CRM_{197}**	**Immunization Schedule (Week)**	**Bleed /Harvest Schedule (Week)**
1	(Aβ17-24-C) LVFFAEDVC + RC529-SE	0, 4, 12	6, 12, 13
2	(Aβ16-23-C) KLVFFAEDC + RC529-SE	0, 4, 12	6, 12, 13
3	(Aβ18-25-C) VFFAEDVGC + RC529-SE	0, 4, 12	6, 12, 13
4	(AβC-17-24) CLVFFAEDV + RC529-SE	0, 4, 12	6, 12, 13
5	(AβC-16-23) CKLVFFAED + RC529-SE	0, 4, 12	6, 12, 13
6	(AβC-18-25) CVFFAEDVG + RC529-SE	0, 4, 12	6, 12, 13
7	(Aβ18-24-C) VFFAEDVC + RC529-SE	0, 4, 12	6, 12, 13
8	(Aβ17-23-C) LVFFAEDC + RC529-SE	0, 4, 12	6, 12, 13
9	(Aβ1-7-C) DAEFRHD + RC529-SE	0, 4, 12	6, 12, 13
10	PBS	0, 4, 12	6, 12, 13

24. The procedures for in vitro stimuli for ELISPOT assays were as follows: Aβ1-42 peptides were synthesized by using the concept of Solid Phase Peptide Synthesis (SPPS) utilizing 9-fluorenylmethoxy-carbonyl (Fmoc) chemistry. Peptide purity was determined by using HPLC and mass spectrophotometry analysis. Stock peptides were made in concentrations of 10 to 20 mg/mL in 100% DMSO, aliquoted, and kept frozen at

-20°C. Aβ1-42 peptides employed in the ELISPOT assays were used at equal molar concentrations (2 μM/well). Aβ1–42 peptides used in this study were obtained from New England Peptide (Gardner, MA).

25. The procedures for enzyme-linked immunosorbent spot (ELISPOT) assay for the detection of IFN-γ secreting cells were as follows: Enzyme-Linked Immunosorbent Spot (ELISPOT) Assay is a common method for monitoring immune responses in humans and animals. ELISPOT assays were originally developed to enumerate B cells secreting antigen-specific antibodies, and have subsequently been adapted for various tasks, especially the identification and enumeration of cytokine-producing cells at the single cell level. In brief, at appropriate conditions the ELISPOT assay allows visualization of the secretory product of individual activated or responding cells. Each spot that develops in the assay represents a single reactive cell. Thus, the ELISPOT assay provides both qualitative (type of immune protein) and quantitative (number of responding cells) information. ELISPOT assays were conducted to enumerate spleen cells secreting IFN-γ after a 20- to 24-hour *in vitro* incubation with the stimuli, human Aβ1-42, CRM_{197} (positive control) and media (negative control). IFN-γ belongs to a class of small (15–28 kD) protein and glycoprotein cytokines (15–28 kD) produced by T cells, fibroblasts, and other cells in response to viral infection and other biologic and synthetic stimuli. IFN-γ binds to specific receptors on cell membranes. Their effects include inducing enzymes, suppressing cell proliferation, enhancing the phagocytic activity of macrophages, and augmenting the cytotoxic activity of T lymphocytes. IFN-γ is a good indicator for the measurement of T-cell response. Kits containing pre-coated plates, assay reagents, and buffers were obtained from BD Biosciences (San Diego, CA). All methods were carried out according to manufacture instructions. All ELISPOT plates were scanned and analyzed using the ImmunoSpot® Series 1 Analyzer (Cellular Technology Ltd.; Cleveland, OH). The frequency of Spot Forming Cells (SFC) was estimated from the number of spots in the wells, and is expressed as SFC/10^6 cells per well. A positive response was defined as having at least twice the number of SFC of that of the non-stimulus media control and having >50

SFC/10^6 cells after media subtraction. Media wells with a background of >120 SFC/10^6 cells were discarded and not included in the analysis.

26. Table 2 represents the ELISPOT assay responses for spleen cells secreting IFN-γ using the standard criteria as previously described. Mice immunized with central epitope Aβ conjugates including Aβ16-23 (group 2, shaded) did not mount any Aβ specific IFN-γ responses above background levels (media control). All groups displayed positive in vitro responses to positive control CRM_{197} except the PBS control (group 10) which did not mount a response to CRM_{197}.

Table 2: IFN-γ ELISPOT Assay Results

In vitro Stimuli	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**	**Group 7**	**Group 8**	**Group 9**	**Group 10**
	Aβ17-24-C	Aβ16-23-C	Aβ18-25-C	AβC-17-24	AβC-16-23	AβC-18-25	Aβ18-24-C	Aβ17-23-C	Aβ1-7-C	PBS
Aβ1-42	2	2	0	0	0	3	0	2	0	0
CRM_{197}	118	280	183	148	277	382	467	232	92	0
Media	0	0	0	2	2	0	2	0	2	5

Values represent SFC / 10^6 spleen cells (Media background has been subtracted is displayed for reference only)

27. In the sixth experiment, I studied the binding of the antisera resulting from immunization with Aβ16-23 to Aβ oligomers.

28. Polyclonal anti-Aβ antibodies were prepared as follows: Anti-Aβ polyclonal antibodies were prepared from blood collected from female balb/c mice that have been immunized with Aβ16-23 conjugated with CRM_{197} plus either adjuvant QS-21 or 529SE, on weeks 0, 4 and 8 via subcutaneous route. Samples were collected via retro-orbital bleeds on weeks 2, 6 and 10 and animals were euthanized by CO2 inhalation after the week 10 bleed. Serum was prepared and the sera were pooled. The antibody fraction was purified using two sequential rounds of precipitation with 50% saturated ammonium sulfate. The final precipitate was dialyzed against PBS and tested for endotoxin. The level of endotoxin was less than 1 endotoxin units/mg (EU/mg).

29. Synthetic Aβ Oligomers were prepared as follows: The Aβ oligomers and monomers present in an Aβ preparation can be determined or visualized by a wide variety of techniques such as immunoprecipitation and Western blot analysis. Aβ oligomers have molecular weights of, for example, about 8 kDa, about 12 kDa, about 16 kDa or about 20 kDa for trimers, tetramers or pentamers, respectively. Aβ oligomers and monomers can consist of any full length Aβ peptide, for example, Aβ1-42 or Aβ1-40, or combinations thereof. Preparation of synthetic Aβ oligomers can be prepared by dissolving lyophilized Aβ1-42 peptides in a solvent to de-aggregate the peptides and produce a solution of substantially unaggregated Aβ peptides. The Aβ peptides are then recovered from the solution and incubated in a culture media to produce a preparation of synthetic Aβ oligomers. The detailed protocol for the preparation of synthetic Aβ oligomers is as follows. Lyophilized Aβ1-42 peptide was dissolved in 100% hexafluoroisopropanol (HFIP) to a final concentration of 100μM and separated into aliquots in microfuge tubes, permitting the peptide to assume a more uniform, unaggregated conformation. HFIP has been shown to break down β-sheet structure, disrupt hydrophobic forces in aggregated amyloid preparations, and promote α-helical secondary structure. The HFIP was removed by both evaporation for 24 hrs in a chemical hood and lyophilization, to remove residual HFIP, and to yield an Aβ peptide residue. The Aβ peptide residue was stored desiccated at –20°C for later use in preparing the aggregated Aβ preparation or used immediately. To use the peptide, the Aβ peptide was resuspended in dimethyl sulfoxide (DMSO) and added to Ham's F-12 (phenol red free) culture media to bring the peptide to a final concentration of 100μM. The resultant solution was then incubated at 4°C for 24 hrs to produce the Aβ preparation. An Aβ preparation, as prepared aforementioned, was treated with peroxynitrite in the presence of sodium hydroxyide (NaOH). Peroxynitrite crosslinks tyrosines (*i.e.* Aβ contains one tyrosine at position 10) and is predicted to stabilize the preparation.

30. The procedures for preparing SDS-PAGE gels for western blotting were as follows: Samples of Aβ preparations were diluted in sample buffer (~1-5μg per lane) and separated by SDS-PAGE on a 12% NuPAGE gel. The protein was transferred to

nitrocellulose membranes, the membranes were microwaved in PBS for 2 minutes, allowed to soak in the hot PBS for 3.5 minutes, and then microwaved again for 5 minutes. The membranes were blocked overnight at 4°C. in blocking buffer (LiCor; Odyssey Blocking Buffer). Membranes were incubated with the primary antibody diluted, as indicated in Fig. 5, in Blocking Buffer + 0.1% Tween 20, overnight at room temperature. The primary antibodies are 6E10 (binds to eptiopes within Aβ6-10), 3D6 (binds to eptiopes within Aβ1-5), 266 (binds to eptiopes within Aβ13-28), TY 11/15 (an unrelated IgGl antibody) and mouse anti-sera collected from immunization of (i) Aβ16-23 conjugated with CRM_{197} plus the adjuvant QS-21 and (ii) Aβ16-23 conjugated with CRM_{197} plus the adjuvant 529SE. After washing membranes in 4 x 5 minutes in PBS + 0.1% Tween 20, membranes were incubated with secondary antibody Alexa 680 anti-mouse IgG. Membranes were washed 4 x 5 minutes in PBS + 0.1% Tween 20 and imaged in Licor's Odyssey Infrared Imaging System. Molecular mass was estimated by Licor's molecular weight markers.

31. The results show that the mouse monoclonal antibodies, 6E10, 3D6, and 266, used as positive controls here, recognized the soluble Aβ1-42 monomer (lower band) as well as higher-ordered oligomeric Aβ species (*i.e.* trimer, tetramer) (Figure 5). The irrelevant antisera TY 11/15 served as a negative control and displayed little to no reactivity to Aβ. In contrast, the antisera resulting from immunization with Aβ16-23 conjugated with CRM197 plus either adjuvant QS21 or 529SE reacted with both monomer and higher-ordered oligomeric Aβ species, with stronger reactivity being displayed by those treated with QS21 compared to those of 529SE (1/5000 dilution resulted in greater intensity bands for the former sample versus latter sample at the same dilution), which is consistent with the calculated average titer (*i.e.* QS21=28,661 vs. 529SE=17,383). These data indicate that the mouse anti-sera to Aβ16-23 recognize both monomer and higher-ordered oligomeric Aβ species.

32. I interpret these data as showing that Aβ16-23 is surprisingly advantageous for treatment or effecting prophylaxis of Alzheimer's disease.

33. All statements made of my own knowledge are true and statements made on information or belief are believed to be true. Further, these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: _____ _____

Jack Steven Jacobsen, Ph.D.

LEGAL02/32808730v1

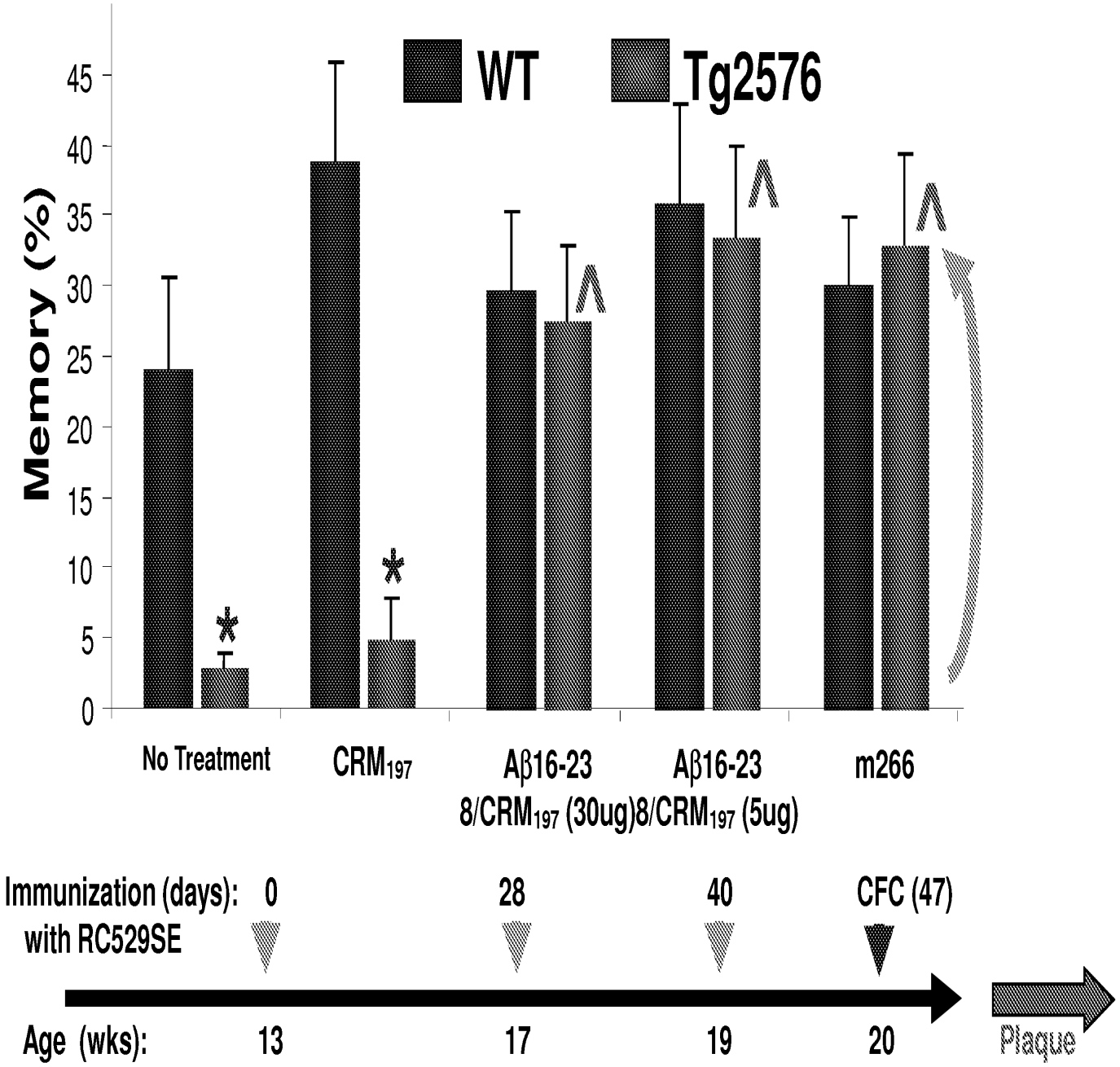


Fig. 1: Cognitive Studies. Reversal of cognitive deficits in Tg2576 mice through active immunotherapy with Aβ16-23/CRM_{197}.

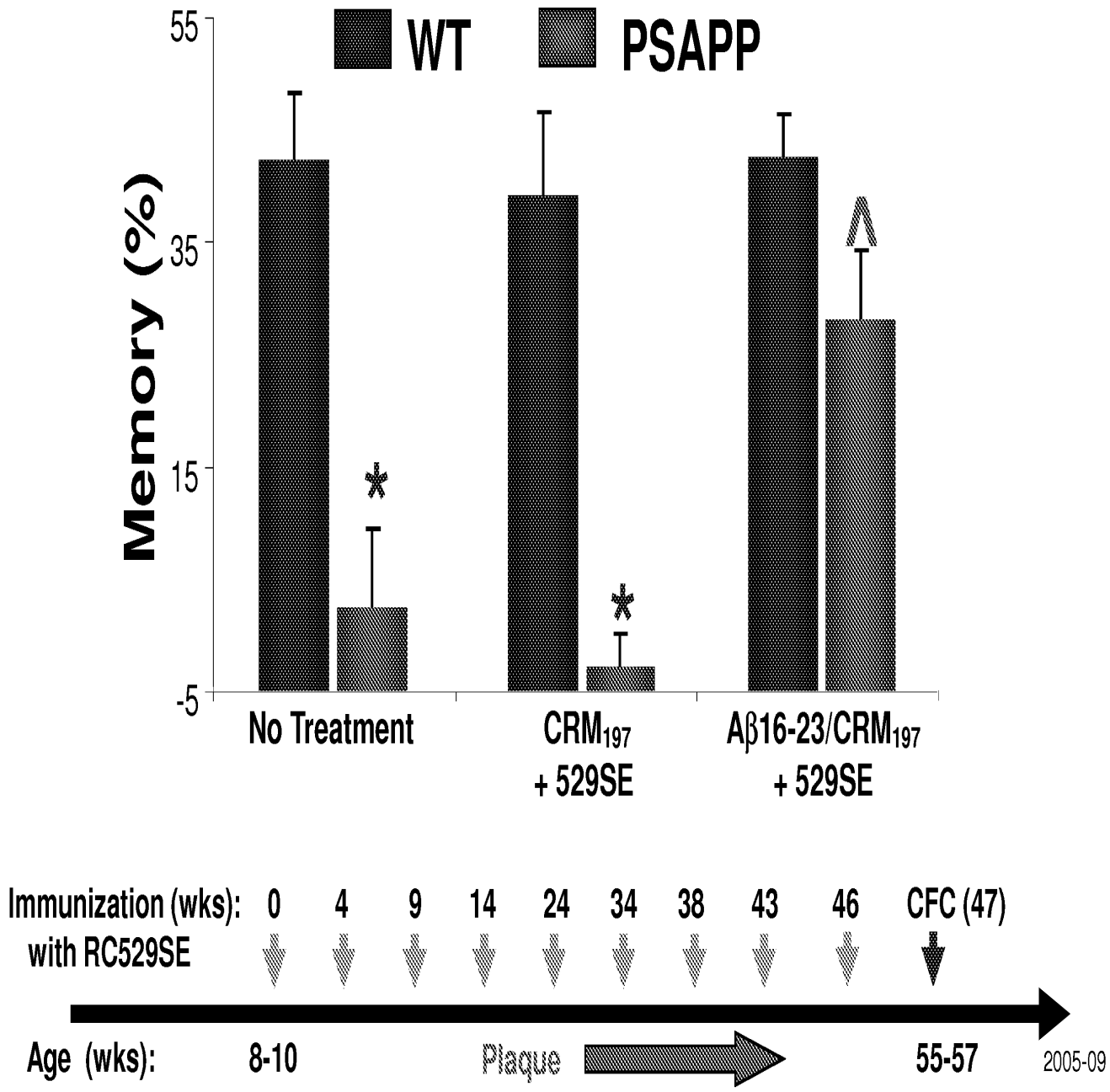


Fig. 2: Cognitive Studies. Prevention of cognitive deficits in PSAPP mice through active immunotherapy with Aβ16-23/CRM_{197} plus adjuvant 529SE.

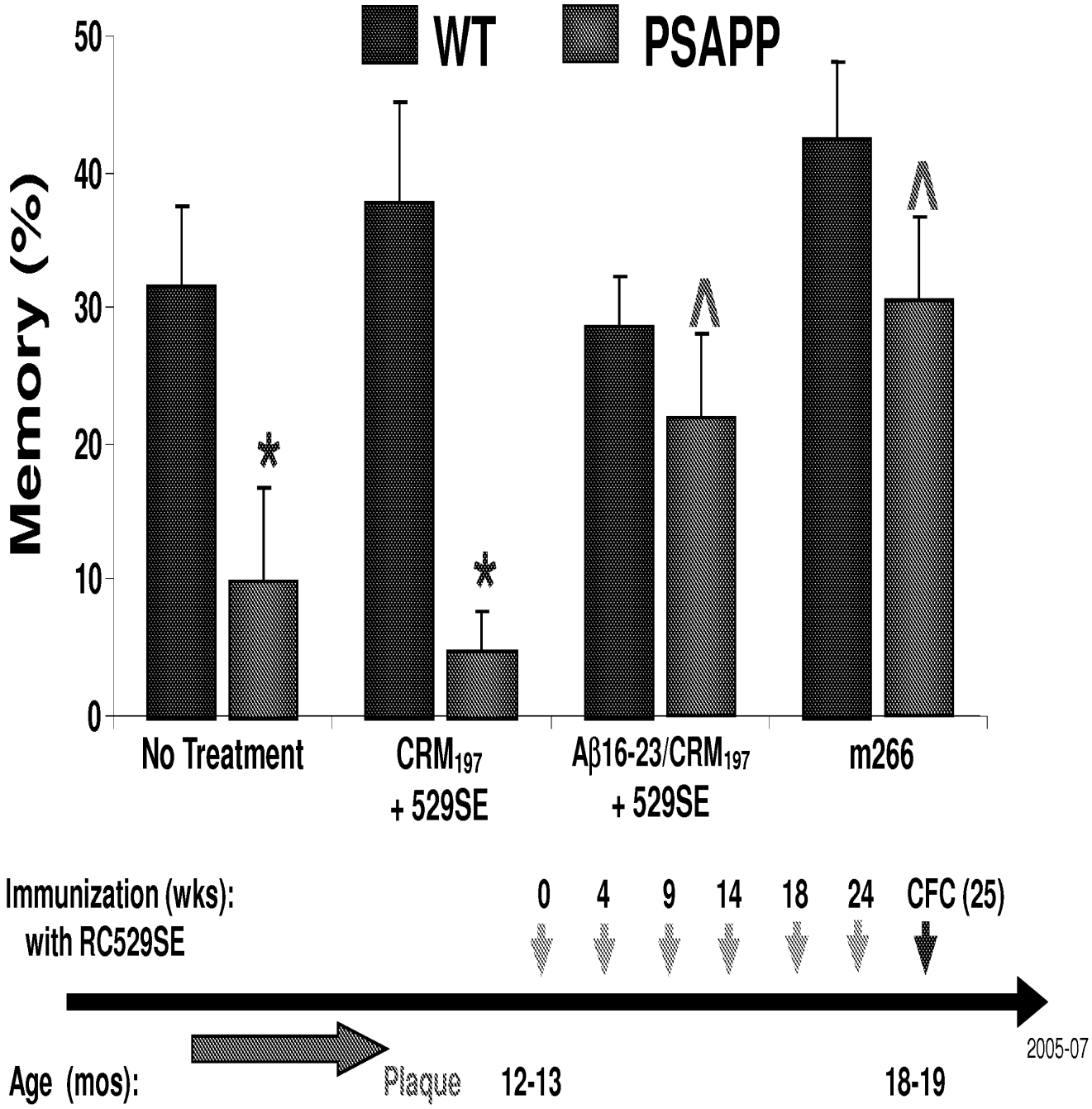
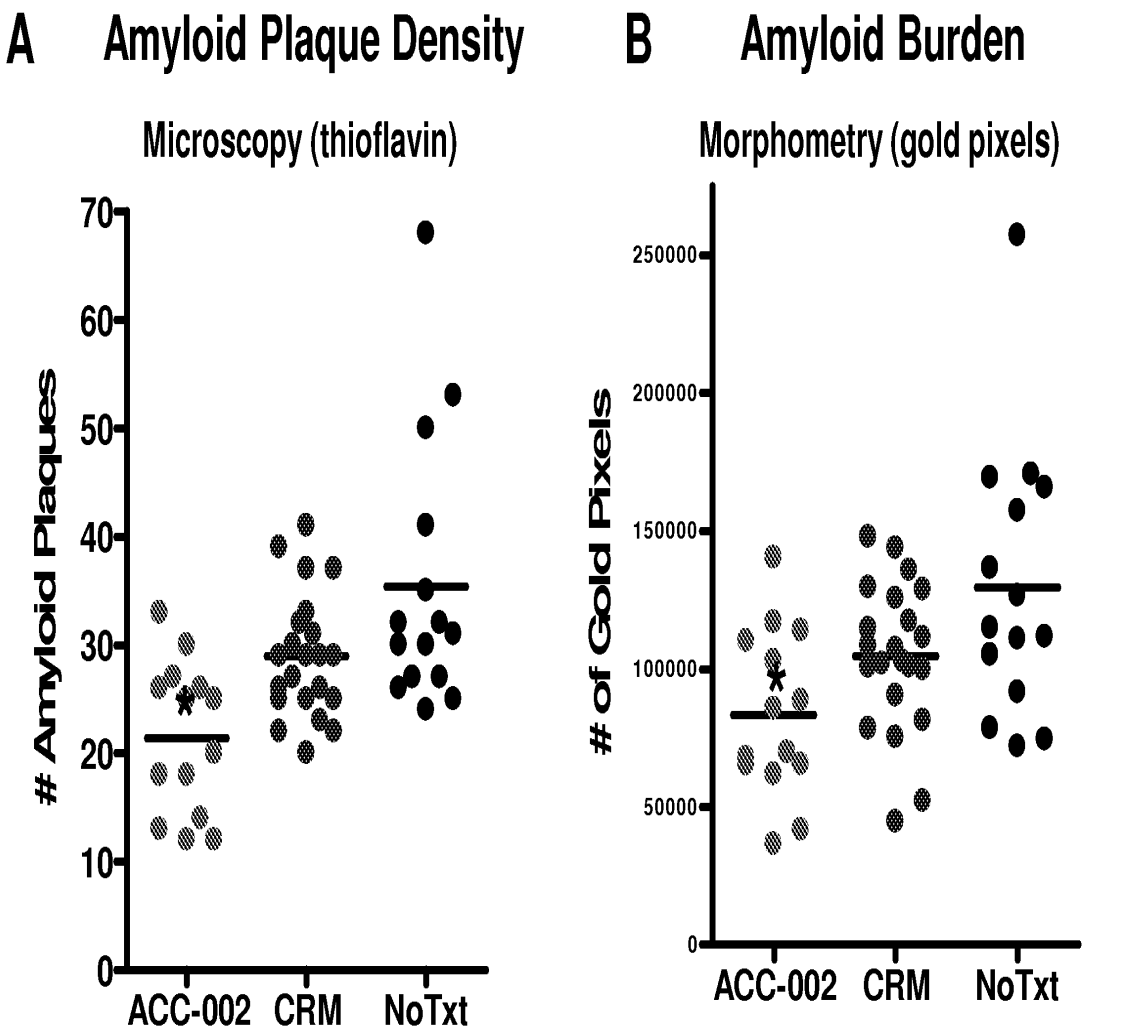


Fig. 3: Cognitive Studies. Treatment of cognitive deficits in PSAPP mice through active immunotherapy with Aβ16-23/CRM_{197} plus adjuvant 529SE.



Treatment	Aβ16-23/CRM_{197}	CRM_{197}	No Txt
Average	21.4	29.0	35.4
% Reduction	39.7	18.2	0
p Value (*M-W)	0.002	0.841	

Aβ16-23/CRM_{197}	CRM_{197}	No Txt
83351	104507	129512
35.6	19.3	0
0.005	0.134	

Fig. 4: Immunization of young PSAPP mice with Aβ16-23/CRM_{197} reduces amyloid plaque formation.

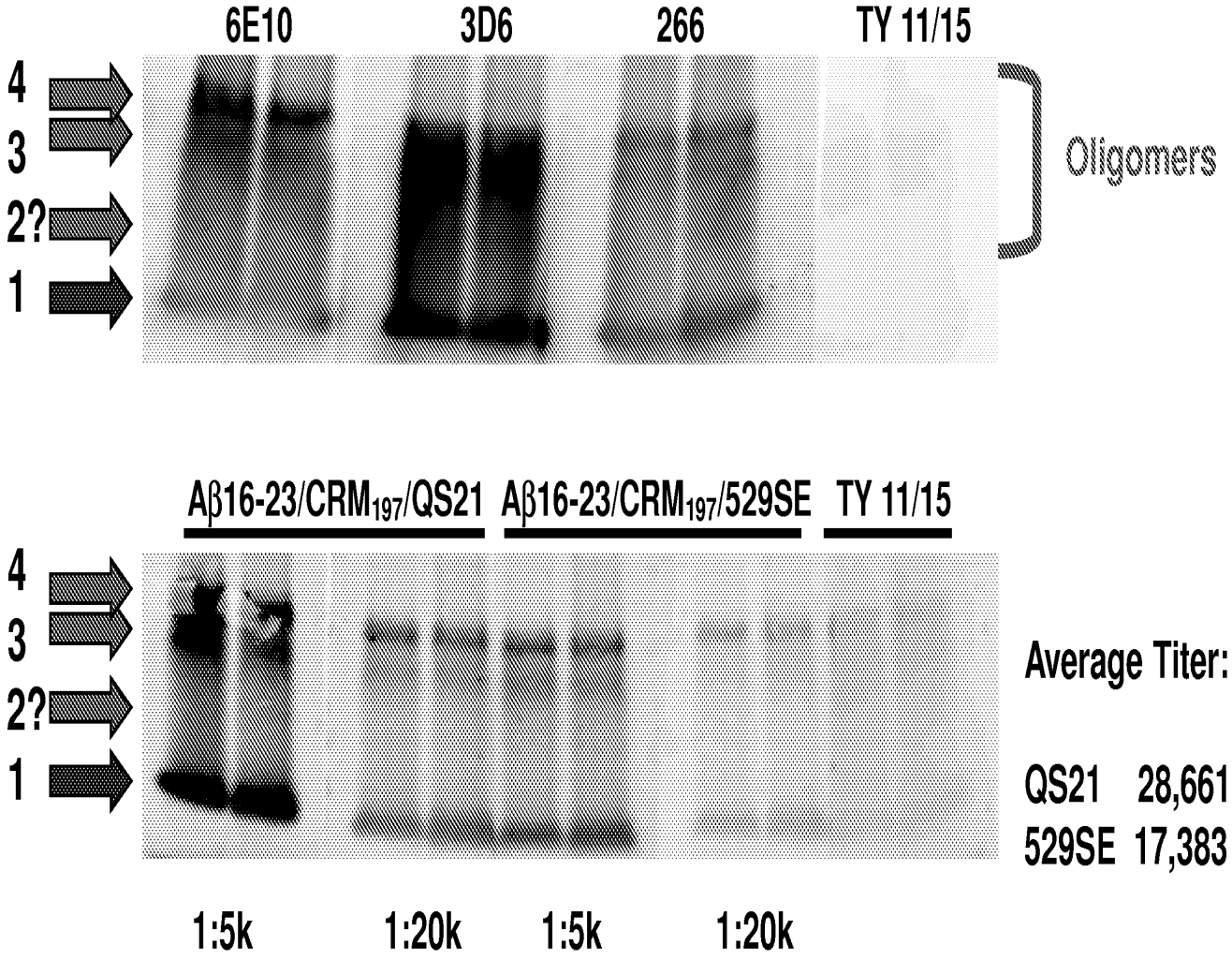


Fig. 5: Aβ16-23/CRM_{197} binds to Aβ oligomer

CURRICULUM VITAE

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History:

Wyeth Research	**07/1995 - Present**
Princeton, NJ, USA	
Associate Director, Neurodegeneration Research	**08/2001 - Present**
Team Leader, Alzheimer's Disease Amyloid Programs	**07/1995 - Present**
Lederle Laboratories, American Cyanamid	**05/1988 - 06/1995**
Pearl River, NY, USA	
Team Leader, Alzheimer's Disease Program	**07/1992 - 06/1995**
Senior Scientist II, Alzheimer's Disease Program	**01/1991 - 06/1992**
Senior Scientist I, Alzheimer's Disease Program	**12/1989 - 12/1990**
Postdoctoral Scientist, Alzheimer's Disease Program	**05/1988 - 11/1989**

1995-Pres. Wyeth Neuroscience, Wyeth, Princeton, NJ.
Research Interests: Disease-modifying strategies for the treatment of patients with Alzheimer's Disease.

1988-1995 Central Nervous System Biological Research Department, Medical Research Division, Lederle Laboratories, American Cyanamid Co., Pearl River, NY.
Research Interests: Molecular mechanisms of Alzheimer's Disease: Expression and processing of Amyloid Precursor Proteins.
1988-1989 Postdoctoral Scientist (Molecular Neurobiologist & Biochemist)
1989-1995 Senior Research Scientist II (Molecular Neurobiologist)

1979-1983 Department of Dermatology, New York University School of Medicine, NYU Medical Center, NY, NY.
Research Interests: Identification & purification of human melanoma-associated cell-surface proteins and the development of a vaccine for immunotherapy.
1979-1980 Research Technician I
1980-1981 Research Technician II
1981-1982 Senior Research Technician

Education:

Graduate, Ph.D. in molecular genetics, Dr. M. Zafri Humayun, Dept. of Microbiology and Molecular Genetics, Graduate School of Biomedical Sciences, University of Medicine and Dentistry-New Jersey Medical School, Newark, NJ, 1983 - 1988.

Graduate, M.S. in biochemistry, Dr. John R. Keller, Department of Biology, Seton Hall University, South Orange, NJ, 1977 - 1979.

Undergraduate, B.S. in biology, Department of Biology, Seton Hall University, South Orange, NJ, 1974 - 1977.

Invited Lectures:

1989 **"Molecular Mechanisms of Amyloid Peptide Precursor in Alzheimer's Disease."** New Jersey Neuropsychopharmacology Society Annual Symposium, Princeton University, Princeton, NJ.

1989 **"Alzheimer's Disease and the Molecular Mechanisms of Amyloid Peptide Precursor Expression."** Graduate Biology Seminar Series, Department of Biology, Seton Hall University, South Orange, NJ.

1990 **"Expression of Amyloid Peptide Precursor Expression and deposition of β-amyloid peptide in Alzheimer's Disease."** Department of Biology, William Paterson College, Wayne, NJ.

1992 **"Processing of β-amyloid peptide in Alzheimer's Disease."** Department of Biology, William Paterson College, Wayne, NJ.

1993 **"Molecular Mechanisms of Alzheimer's Disease: Therapeutic approaches to reduce the formation of β-amyloid peptide."** Graduate Biology Seminar Series, Department of Biology, Seton Hall University, South Orange, NJ.

1994 **"Alzheimer's Disease and β–amyloid peptide: Molecular Mechanisms to Block the Formation of β–Amyloid Peptide."** Dept. of Molecular Biology and Molecular Genetics, University of Medicine and Dentistry-New Jersey Medical School, Newark, NJ.

1995 **"Alzheimer's Disease and β–amyloid peptide: Molecular Mechanisms to Drug Discovery."** Dept. of Environmental Medicine, NYU School of Medicine, Tuxedo, NY.

1995 **"Therapeutic Strategies to Reduce Formation of β–amyloid peptide in**

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1999 "Advances in Understanding the Molecular Mechanisms of Alzheimer's Disease." Depts. of Environmental Medicine and Biology, NYU School of Medicine, NY, NY.

2000 "Alzheimer's Disease: Development of Protease Inhibitors as Aβ-Lowering Agents." American Home Products Corporation, Senior Managers Symposium, Baltimore, MD.

2001 "Alzheimer's Disease: Current and Emerging Therapeutic Strategies." *Medicinal Chemistry*, Gordon Research Conferences, Colby-Sawyer College, New London, NH.

2001 "Strategic Approaches for the Treatment of Alzheimer's Disease." Lecture series in Medicinal Chemistry, Associated Colleges of the Chicago Area (ACCA), Morton Arboretum, Lisle, IL.

2004 "Alzheimer's Disease And Emerging New Disease-Modifying Therapeutic Strategies." The 2004 Robert S. Rozman Memorial Symposium, Delaware Valley Drug Metabolism Discussion Group, Langhorne, PA.

2005 "Anti-Amyloid Approaches for the Treatment of Alzheimer's Disease." 7TH International Neurodegeneration In Alzheimer's Disease, Parkinson's Disease and Related Disorders, Strategic Research Institute, Princeton, NJ. (April 2005)

2005 "Alzheimer's Disease: The Next Generation of Symptomatic and Disease Modifying Therapies." Bio2005, Philadelphia, PA. (June 2005)

2005 "Disease-Modifying Therapeutic Approaches for the Treatment of Alzheimer's Disease." Therapeutic Strategies Against Neurodegenerative Conditions, Boston, MA. (Oct. 2005)

Professional Membership, Review Committees and Appointments:

1991-present Society for Neuroscience
Chicago, Il

1993-present *AD HOC* Review Committee Member
Alzheimer's Disease Center Cores (ADCCs)
National Institute on Aging (NIA/NIH), Bethesda, Md.

1994-present External Peer Review Committee Member
UMDNJ and Allied Health Services Foundation
University of Medicine and Dentistry-New Jersey Medical School, New Brunswick, NJ.

1995-present Adjunct Associate Professor (Lecturer in Neurobiology and Neurotoxicology; Graduate Student Thesis Committee Member)
New York University School of Medicine, NYU Medical Center, NY, NY.

1997-present Review Board Committee Member,
Medical and Scientific Advisory Council,
Alzheimer's Association, Inc., Chicago, Il,

2003-Present *Current Alzheimer Research*, Editorial Board (Bentham Press)

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